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(54) **Collagen-containing sponges as drug delivery for proteins.**

(57) Collagen-containing sponges comprising an absorbable gelatin sponge, collagen, and an active ingredient are disclosed as well as methods of enhancing wound healing if external and internal wounds using such sponges are disclosed.

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BACKGROUND OF THE INVENTION

The present invention relates to collagen containing sponges for improved sustained release delivery of pharmaceuticals.

Collagen has been used previously as a component in pharmaceutical sponges. Artandi [U.S. Patent 3,157,524, issued November 17, 1964], disclosed a sponge comprised of acid treated swollen collagen. Oluwasanmi et al. [*J. Trauma* 16:348-353 (1976)] disclosed a 1.7 millimeter thick collagen sponge that is crosslinked by glutaraldehyde. Collins et al. [*Surg. Forum* 27:551-553 (1976)] disclosed an acid-swollen collagen sponge that is crosslinked by glutaraldehyde. Berg et al. [U.S. Patent 4,320,201, issued March 16, 1982], disclosed a swollen sponge of high collagen purity produced by enzymatically degrading animal hides, digesting the mass in alkali or acid, mechanically comminuting the mass to produce specified lengths of collagen fibers, and crosslinking the fibers. Berg et al. [U.S. Patent 4,837,285, issued June 6, 1989], disclosed porous beads that have a collagen skeleton of 1 to 30 percent of the bead volume. These beads are useful as substrates for cell growth. Doillon et al. *[Scanning Electron Microscopy* III:1313-1320 (1984)], Doillon and Silver *[Biomaterials* 7:3-8 (1986)], and Doillon et al. *[Biomaterials* 8:195-200 (1987)], disclosed the growth of cells in collagen sponges with pore sizes of from about 60 to 250 μm. Some of the sponges also contained hyaluronic acid and/or fibronectin.

In addition, collagen has been used as a component in salves [PCT Patent Application WO 86/03122, published June 5, 1986]. Collagen has also been used for wound healing in conjunction with electrical currents [Silver and Dunn, U.S. Patent 4,937,323, issued June 26, 1990].

In addition, membranes containing collagen have been used in the prior art. Abbenhaus et al., *Surg. Forum* 16:477-478 (1965) disclosed collagen films of 2 to 3 millimeter thickness that was produced by heating and dehydrating collagen extracted from cow hides. Chu disclosed non-chemically crosslinked collagen implants produced by compression, which are useful for sustained drug delivery [European Patent Application 187014, published July 9, 1986; U.S. Patent 4,600,533, issued July 15, 1986; U.S. Patent 4,655,980, issued April 7, 1987; U.S. Patent 4,689,399, issued August 25, 1987; and PCT Patent Application WO 90/00060, published June 28, 1989]. Cioca [U.S. Patent 4,412,947, issued November 1, 1983], disclosed an essentially pure collagen sheet made by freeze drying a suspension of collagen in an organic acid. Kuroyanagai et al. [European Patent Application 167828, published January 15, 1984; U.S. Patent 4,642,118, issued February 10, 1987], disclosed an artificial skin composed of two layers: collagen and a poly-alpha-amino acid. Berg et al. [U.S. Patent Application 4,841,962, issued June 27, 1989], disclosed a wound dressing composed of three layers: an adhesive, a cross-linked collagen matrix, and a multilayer polymer film. Holman, U.S. Patent 4,950,699, issued August 21, 1990, disclosed a wound dressing consisting of less than 10% collagen mixed with an acrylic adhesive. Cioca et al., British Patent 1,347,582, disclosed a collagenic wound dressing consisting of a freeze dried polydisperse collagen mixture.

Steffan et al., European Patent 069260, published January 12, 1983, disclosed a collagen insert consisting of high purity native collagen. Zimmerman et al. [U.S. Patent 4,453,939, issued June 12, 1984], disclosed a wound healing composition containing collagen coated with fibrinogen, factor XIII fibrinigen, and/or thrombin. Leibovich et al. [U.S. Patent 4,808,402, issued February 1989], disclosed a composition for treating wounds comprising collagen, bioerodible polymer, and tumor necrosis factor. Yannas and Burke [*J. Biomed. Mat. Res.* 14:68-81 (1980)], have reviewed the design of artificial skin, some examples of which contain collagen. Chvapil et al., *Int. Rev. Connect. Tissue Res.* 6:1-61 (1973), particularly at pages 51 to 52; and Pachence et al., *Med. Device and Diag. Ind.*, 9:49-55 (1987), disclose various uses of collagen, including as a drug delivery vehicle.

Most of the previously utilized collagen containing sponges have been used as substrates or skeletons for the growth of cells at a wound site and have not been used for delivery of pharmaceutical agents. The present invention provides a much desired improvement in pharmaceutical sponges by providing for a steady, continuous and sustained release of therapeutic agents over an extended period of time

SUMMARY OF THE INVENTION

The present invention relates to a collagen-containing sponge comprising an absorbable gelatin sponge, collagen, and an active ingredient. Preferably, the absorbable gelatin sponge is a crosslinked gelatin.

Another aspect of the present invention relates to a collagen-containing sponge further comprising one or more of a plasticizer, a stabilizing agent, a drying enhancer or a buffer. Preferably, the plasticizer is selected from polyethylene glycol and glycerol; the stabilizing agent is a sugar selected from the group consisting of mannitol, lactose and glucose; the drying enhancer is an alcohol selected from the group consisting of ethanol, methanol and isopropyl alcohol; and the buffer is a suitable biological buffer. Preferred active ingredients are selected from the group consisting of PDGF, EGF, FGF, PDEGF, PD-ECGF, KGF, IGF-1, IGF-2, TNF, BDNF, CNTF, and NT-3.

Another aspect of the present invention relates to a method of enhancing wound healing of an epider-

mal wound comprising administration of a wound healing effective amount of an active ingredient via a collagen sponge according to another aspect of the present invention.

Another aspect of the present invention relates to a method of enhancing wound healing of an internal wound comprising administration of a wound healing effective amount of an active ingredient via a collagen sponge according to another aspect of the present invention.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows a release profile for PDGF from a dry Gelfoam-Collagen Sponge *in vitro.*

Figure 2 shows a release profile for PDGF from a dry Gelfoam-Collagen Sponge *in vitro* showing first order kinetics.

Figure 3 shows the correlation of PDGF measured by radioisotope, ELISA and PDGF bioassay.

Figure 4 shows a release profile for PDGF from a wet Gelfoam-Collagen Sponge *in vitro.*

Figure 5 shows a release profile for PDGF from a wet Gelfoam-Collagen Sponge *in vitro* showing first order kinetics.

Figure 6 shows the correlation of PDGF measured by a radioisotope, ELISA and PDGF bioassay.

Figure 7 shows a release profile *in vitro* for PDGF from a Gelfoam Sponge without the presence of Collagen.

Figure 8 shows a release profile *in vivo* for PDGF from a Gelfoam-collagen system prepared with 1% collagen solution.

Figure 9 shows a release profile *in vivo* for PDGF from a Gelfoam-collagen system prepared with 8% collagen solution.

Figure 10 shows experimental results obtained by using the rat blood bundle model.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a collagen-containing sponge comprising an absorbable gelatin sponge, collagen, and an active ingredient.

An appropriate absorbable gelatin sponge is crosslinked gelatin, for example Gelfoam (Upjohn, Inc., Kalamzoo, Michigan). The absorbable gelatin sponge can be combined with a collagen and active ingredient by soaking the absorbable gelatin sponge in a solution comprising soluble collagen and active ingredient. Alternately, a predetermined amount of a collagen solution can be transferred on top of the gelatin sponge and the solution can be absorbed.

Soluble collagen is collagen that has an average molecular weight of less than 400,000, preferably having a molecular weight of about 300,000. A particularly suitable soluble collagen is Semex S (Semex Medical Co., Malvern, Pennsylvania). This particular soluble collagen is also advantageous because it is the atelopeptide form of the collagen. Atelopeptide collagen is collagen that is free of telopeptide, which is a peptide located at one end of purified collagen often associated with immunogenicity. A solution of the telopeptide form of collagen can be converted to the atelopeptide form of collagen via hydrolysis using organic acid. Another preferred characteristic of the soluble collagen is that it possesses a minimal amount of crosslinking, i.e., 0.5% or less.

The soluble collagen can be dissolved in a suitable solvent such as water to produce a solution that contains from about 0.5 to about 10% of collagen by weight, preferably from about 1 to about 5% by weight, and more preferably about 2% by weight.

A solution of soluble collagen or a dispersion of collagen fibrils in suspension is used to soak the absorbable gelatin sponge. Typically, a suitable amount of the collagen solution is transferred on top of the absorbable gelatin sponge. In another case, the collagen solution is poured into a container holding the gelatin sponge. Suitable conditions include allowing the soaked sponge to dry at a suitable temperature for a suitable period of time. Generally, the amount of drying time necessary is shorter as the drying temperature is raised. Specifically, a suitable temperature is from about 15°C to 35°C, preferably about room temperature, and a suitable drying time is sufficient time so that the marginal loss of solvent content is essentially zero (e.g., drying time of about an hour to about 10 days, preferably about one to about five days).

To optimize desirable characteristics of a preferred collagen sponge, various additives may be optionally included in the collagen solution. Such desirable characteristics include flexibility, stability, accelerated drying time and a pH compatible with the active ingredient to be utilized.

To improve flexibility, a suitable plasticizer can be used. Suitable plasticizers include polyethylene glycol and glycerol, preferably glycerol. Such plasticizers can be present in an amount from zero to about 100% of the weight of collagen present, preferably from about 10 to about 30% of the weight of collagen present, most preferably about 20% of the weight of collagen present.

To improve the stability of the active ingredient, a suitable stabilizing agent can be used in the film. Suitable stabilizing agents include most sugars, preferably mannitol, lactose, and glucose, more preferably mannitol. Such stabilizing agents can be present in an amount from zero to about 5% of the weight of collagen present, preferably about 1% of the weight of collagen present.

To accelerate drying time for the films, a drying enhancer can be used. Suitable drying enhancers include alcohols, preferably ethanol, methanol and isopropyl alcohol, more preferably ethanol. Such drying

enhancers can be present in an amount from zero to about 50% of the weight of the total solution or suspension, preferably from about 10 to about 30% of the weight of the total solution or suspension, more preferably about 20% of the weight of the total solution or suspension.

To produce a pH that is compatible with a particular active ingredient being used, a suitable buffer can be used in the film. Suitable buffers include most of the commonly known and utilized biological buffers, preferably acetate, phosphate and citrate, more preferably acetate and phosphate. Such buffers can be present in an amount of from about 0.01% to about 2% of the weight of the collagen solution or suspension. A compatible pH is one that maintains the stability of an active ingredient, optimizes its therapeutic effect or protects against its degradation. A suitable pH is generally from about 3 to about 8, preferably about 5 to about 8, and most preferably about neutral pH of from about 7.0 to about 7.5.

Preferred active ingredients are those biological agents which enhance wound healing or regeneration of nerve tissue, particularly recombinant proteins. Such preferred active ingredients include platelet derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet derived epidermal growth factor (PDEGF), platelet derived endothelial cell growth factor (PD-ECGF) keratinocyte growth factor (KGF), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), tumor necrosis factor (TNF), brain derived neurotrophin factor (BDNF), ciliary neurotrophic factor (CNTF) and neurotrophin-3 (NT-3). A preferred active ingredient is PDGF or PD-ECGF, most preferably PDGF. Such active ingredients are present in an amount sufficient to enhance healing of a wound, i.e., a wound healing effective amount. The actual amount of the active ingredient will be determined by the attending clinician and will depend on various factors such as the severity of the wound, the condition of the patient, the age of the patient and any collateral injuries or medical ailments possessed by the patient. Generally, the amount of active ingredient will be in the range of about 1 $\mu g/cm^2$ to 5 mg/cm^2.

The collagen sponges of the present invention are useful as a means of delivering the active ingredient to cells or tissue with which it is in contact. For example, in the treatment of burns or other traumas to the skin, a collagen sponge can be placed on the wound to deliver a suitable active ingredient to the traumatized area. PDGF is a particularly suitable active ingredient for such uses. Collagen sponges can also be used to accelerate healing of surgical wounds. When used in such a way, the sponge can be placed in the surgical incision and stitched into the wound as an interface between the two surgical wound surfaces. Collagen sponges can also be used to deliver neurotrophic factors. When used in such a manner, the collagen sponge can be placed in direct contact with or adjacent to the nerve tissue to be treated with the neurotrophic factor.

EXAMPLES

Example 1: Preparation of a Collagen Sponge.

A piece of gelatin sponge (Gelfoam®:80 x 250 x 10 mm), was rolled out with a metal rod (diameter: 32 mm, length:130 mm),much like dough to make a thin sheet. The resulting thickness of the sheet was 1mm. Wafers were cut out from the thin sheet with a brass punch. Wafers with a large diameter (diameter:18 mm, weight: 1.5 mg) were cut for *in vivo* studies.

In one study, 0.24 ml or 0.40 ml of the collagen solution containing PDGF was pipetted on top of each wafer. In another study, the wafers were soaked in a collagen solution. The collagen solution containing PDGF was prepared as follows. First, a 4% collagen solution was prepared by dissolving 2 g of a soluble collagen (Semex S Semex, Frazer, Pennsylvania) in 50 ml of distilled water at 30-50°C. To 16 ml of the resulting solution, 0.13 ml of glycerol, 2 ml of alcohol, and 2 ml of a PDGF solution (2 mg/ml acetate buffer) was added. Additionally, a trace amount of ^{125}I-PDGF was added to the solution. After the solution was absorbed by each wafer, the wafer was dried overnight to produce a sponge-collagen system containing PDGF.

Example 2: Kinetics of Release from Collagen Sponges.

Release rates of active ingredients from collagen sponges were conducted using Coster Transwell Cells ("Cell") (Costar Co., Cambridge, Massachusetts) as follows. Collagen sponges with ^{125}I-PDGF were produced as described in Example 1, and wafers (1.6 cm diameter) were cut from the films. Each wafer was transferred to a Coster Transwell Cell and placed on top of the polycarbonate membrane. 2.5 ml of the receiver solution (water and 1% bovine serum albumin, or water and 0.25% human serum albumin) was added to the Cell holder. The Cells were set on the solution and the release study was initiated. At specified times, 20 µl of the receiver solution was pipetted out and the same amount of fresh solution was replaced in the receiver solution. The sampling procedure was repeated to get another 20 µl sample. The radioactivity of the sample was measured with a gamma counter (Beckman Instruments, Co., Irvine, California). The concentration of protein in the receiver solution at any given time was calculated based on the radioactivity and confirmed using other methods such as ELISA and ^{3}H-thymidine uptake bioassays. Figures 1 and 2 and 4 and 5 show a typical release profile of PDGF from the wafers. The release profile

from the dry gel (Figures 1 and 2) system was very similar to that of the wet gel (Figures 4 and 5) system where the release study was performed immediately after the absorption of the collagen solution by the gelatin sponge. About 80% of the growth factor was released in 100 hours. Data shown in Figures 3 and 6 indicate that the drying process does not change the activity of the growth factor.

Figure 7 shows the release of PDGF from a Gelfoam sponge without the presence of collagen. In this study the release of PDGF from the Gelfoam was performed. To remove air bubbles trapped inside the membrane while soaking the membrane (thickness: 1 cm) in PDGF solution, the membrane was soaked in water at reduced pressure. The water soaked membrane was equilibrated in PDGF solution. The release rate of PDGF was measured with Costar Transwell cells using ^{125}I-PDGF. The release rate was very fast. Most of the PDGF was released in 12 hours. The results obtained from this study show the combination system of collagen-Gelfoam provides for a steady, continuous and sustained release of therapeutic agents over an extended period of time.

Example 3: Measurement of *In Vivo* Release Rate of Protein From the Collagen-Sponge System.

A. Pre-operative Preparation

Young adult New Zealand White rabbits, weighing approximately 3.0 to 3.5 kg each (M & K Rabbitry, Bentonville, Arkansas) were anesthetized using Rompum® (Farbenfabriken, Bayer, West Germany) as a sedative, followed (10 minutes later) by ketamine (60 mg/kg) and xylaine (5 mg/kg), both administered intramuscularly. Each rabbit's weight was measured and recorded. A small cotton or gauze plug was inserted into both ears of each rabbit, after which the inner surface and outer edges of both ears were shaved using an animal clipper (#40 blade). Commercially available Neet® depilatory cream was then applied to the inner surface of each ear for 10 minutes, after which time it was removed with dry gauze. The inner surface of the ears was wiped with saline-soaked gauze followed by application of a 70% alcohol solution. The dermis of the inner surface on one ear of each rabbit was blanched by infiltration of the ear with a 2% xylocaine solution containing 1:1000 epinephrine (this requires 1.5 to 3.0 mls total volume) using a 30 gauge needle. The infiltrated area was then scrubbed with 3 cycles of betadine followed by the 70% alcohol solution. Where necessary, the ear plugs were replaced with dry plugs at this point.

The rabbits were then transferred to a sterile surgical room. The blanched ear was immobilized on a plexiglass "ear board" (Washington University Medical Center, Division of Technical Services, St. Louis, Missouri) which utilizes two bar clamps, one at the tip and one at the base of the animal's ear, to stabilize the rabbit ear without compromising its blood supply. The animal was draped, and the surgical field (i.e., the inner surface of the blanched ear) sprayed with Betadine and allowed to dry for 3 to 5 minutes.

B. Wounding

Sterile technique was employed throughout the wounding procedure. Using microsurgical instruments, a 6 mm trephine, and a binocular microscope (10x, Zeiss), the surface of the inner ear of each rabbit was scored gently with a 6 mm biopsy punch, and the biopsy site cleared of all tissue and fibers (including the periosreal membrane) down to the level of bare cartilage, using micro-surgical forceps, tenotomy scissors, a blunt edged 2 mm Lempert periosreal elevator, and sterile cotton-tipped applicators. Perichondrium and overlying tissues were removed by dissection. Biopsies in which the cartilage was completely cut through by the punch were not used for experimental purposes. However, partial thickness scores of the cartilage were considered acceptable. The location of any nicks or natural holes in the cartilage was carefully noted and recorded (for reference on the harvest day). Blood was removed from the biopsy site with sterile, cotton-tipped applicators, with care taken to avoid excess blood in the wound. Each completed biopsy was covered with a small piece of saline-soaked gauze. Four viable 6 mm biopsy ulcers were placed on each wounded ear, two on each side of the midline (as defined by the fold in the ear when it was stabilized upon the board). In any event, no more than 5 total biopsies were placed on each ear. The biopsies were positioned a minimum of 1 cm apart.

Upon completion of one ear, the ear was covered with saline-moistened gauze and then taped shut around the gauze to retain moisture until application of PDGF. The second ear was then blanched, scrubbed, immobilized and wounded in the manner as the first ear. Blood was removed from the biopsy site of each second ear and each completed biopsy covered with a small piece of saline-soaked gauze. Upon completion of the second ear, it was covered with saline-moistened gauze until application of PDGF. Any rabbit that showed evidence of recovery from anesthesia at any time prior to this point in the procedure was re-anesthetized with 25 mg/kg ketamine, administered intramuscularly.

C. Application of Active Ingredient to Wounds

Wafers prepared by the procedure of Example I were used for *in vivo* drug release study. Small wafers (diameter: 5mm) were used for this experiment. Each wafer was inserted in a wound site (diameter: 6mm) and a piece of Tagaderm® was used to make sure

that the wafer remained in the wound site.

The rabbits were allowed to recover from anesthesia under the observation of the investigator performing the surgery. Upon recovery, a plastic neck collar (Canine Center, St. Louis, Missouri) extending approximately 15 to 25 cm outward was placed around each rabbit's neck to prevent the rabbit from disrupting the wounds or dressings. The rabbits were returned to an isolation cage where they were maintained until harvest.

D. Measurement of the Protein Release from the Collagen-Gelatin Sponge System

At selected times, several wafers were taken from the wound sites. New pieces of Tagaderm® were used to cover the wound sites where the wafers were removed. The wafers were collected in test tubes. When all the wafers were recovered, the radioactivity of each wafer was counted. The amount of PDGF released at a given time was calculated from the radioactivity of the remaining wafer. Figure 8 shows the release profile of PDGF from the collagen-Gelfoam system which was prepared with 1% collagen solution. After 49 hours, about 62% of PDGF was released. Figure 9 shows the release profile of PDGF from the collagen-Gelfoam system which was prepared with 8% collagen solution. This solution was obtained by dissolving the soluble collagen in distilled water at 60°C. About 73% of PDGF was released after 49 hours.

Example 4: Effect of Collagen-Containing Sponges on New Tissue Growth as Measured by the Rat Blood Bundle Model.

In 30 Lewis rats (125-150 grams), the left tibialis posterior and its parent femoral arterio-venous bundle were dissected from the ankle up to the inguinal ligament. The bundles were sandwiched between two collagen disks 1.6 cm in diameter and placed inside a spoon-shaped silastic mold. Disks from Group A were based on collagen-Gelfoam system. Each disk in the group contained about 125 µg of PDGF. Disks from Group B were based on soluble collagen. Each disk in the group contained about 125 µg of PDGF. Disks from Group C were made of soluble collagen without any PDGF and therefore were used as controls. All the animals were sacrificed at day 30. The contents of the molds were examined grossly, and with a digitizing computer and 3-D reconstruction, histomorphometrically to determine the volume of tissue generated.

Figure 10 shows the generation of new tissue volumes of three different groups. Substantial increase in new tissue volume was observed in the animals treated with the collagen-Gelfoam system containing 125 µg of PDGF. In the previous experiments using a soluble collagen system with the same animal model, it was found that wafers based on soluble collagen containing PDGF increased the volume of new tissue significantly after implantation between day 10 and day 15. This was especially evident with high doses of PDGF (120 µg/wafer). The study also showed that 30 days after implantation, the new tissue regressed. The current experiments demonstrate that the collagen-Gelfoam system works well for the generation and/or maintenance of new tissue volume for several weeks.

The features disclosed in the foregoing description, in the following claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Claims

1. A collagen-containing sponge comprising an absorbable gelatin sponge, collagen, and an active ingredient.

2. A collagen-containing sponge according to claim 1 wherein said absorbable gelatin sponge is a crosslinked gelatin.

3. A collagen-containing sponge according to claim 2 wherein said crosslinked gelatin is Gelfoam.

4. A collagen-containing sponge according to claim 3 further comprising a plasticizer.

5. A collagen-containing sponge according to claim 4 wherein said plasticizer is selected from polyethylene glycol and glycerol.

6. A collagen-containing sponge according to claim 4 further comprising a stabilizing agent.

7. A collagen-containing sponge according to claim 6 wherein said stabilizing agent is a sugar selected from the group consisting of mannitol, lactose and glucose.

8. A collagen-containing sponge according to claim 6 further comprising a drying enhancer.

9. A collagen-containing sponge according to claim 8 wherein said drying enhancer is an alcohol selected from the group consisting of ethanol, methanol and isopropyl alcohol.

10. A collagen-containing sponge according to claim 8 further comprising a buffer.

11. A collagen-containing sponge according to claim

1 wherein said active ingredient is selected from the group consisting of PDGF, EGF, FGF, PDEGF, PD-ECGF, KGF, IGF-1, IGF-2, TNF, BDNF, CNTF, and NT-3.

12. A collagen-containing sponge according to claim 10 wherein said active ingredient is selected from the group consisting of PDGF, EGF, FGF, PDEGF, PD-ECGF, KGF, IGF-1, IGF-2, TNF, BDNF, CNTF, and NT-3.

13. A collagen film according to claim 11 wherein said active ingredient is PDGF.

14. A collagen film according to claim 12 wherein said active ingredient is PDGF.

15. A method of enhancing wound healing of an epidermal wound comprising administration of a wound healing effective amount of an active ingredient via a collagen sponge according to claim 1.

16. A method of enhancing wound healing of an epidermal wound comprising administration of a wound healing effective amount of an active ingredient via a collagen sponge according to claim 10.

17. A method of enhancing wound healing of an internal wound comprising administration of a wound healing effective amount of an active ingredient via a collagen sponge according to claim 1.

18. A method of enhancing wound healing of an internal wound comprising administration of a wound healing effective amount of an active ingredient via a collagen sponge according to claim 10.

Fig 1 PDGF Release from Gelfoam-Collagen System

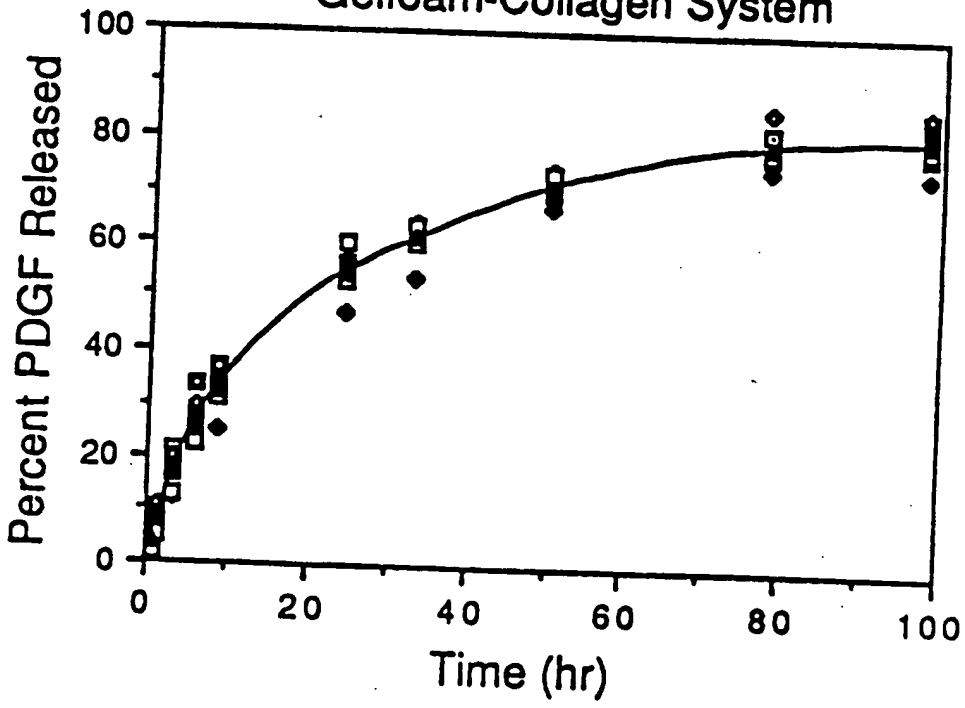


Fig 2 PDGF Release from Gelfoam-Collagen System

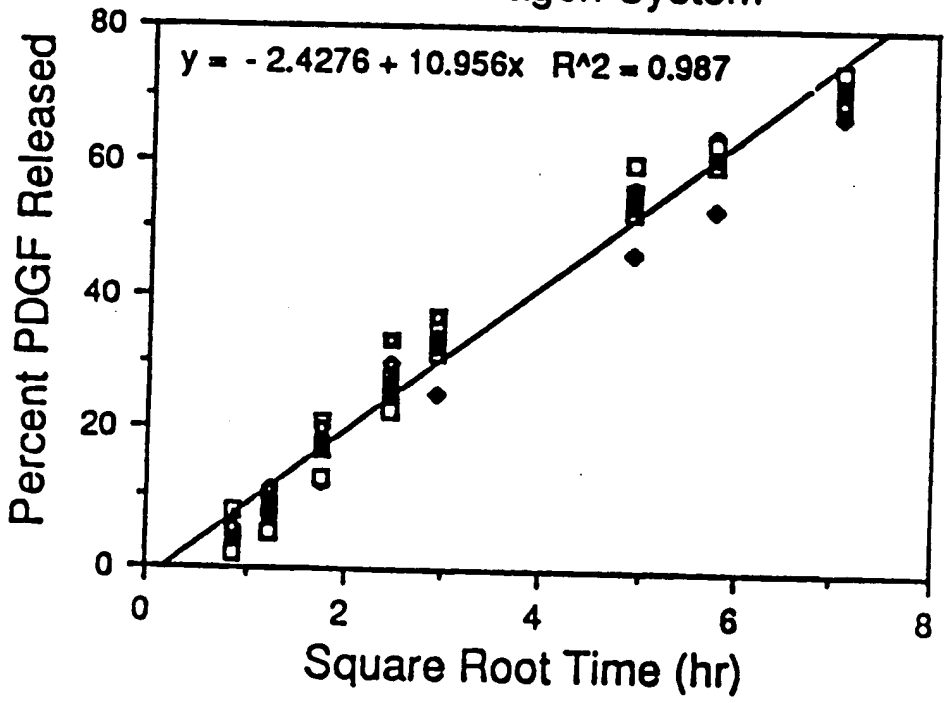
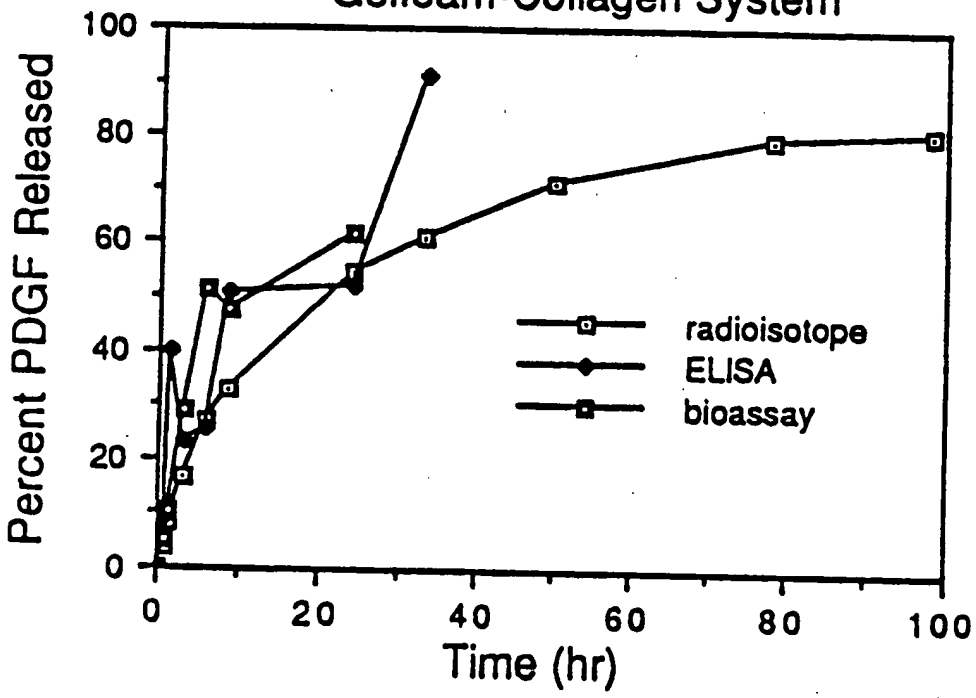


Fig 3 PDGF Release from Gelfoam-Collagen System



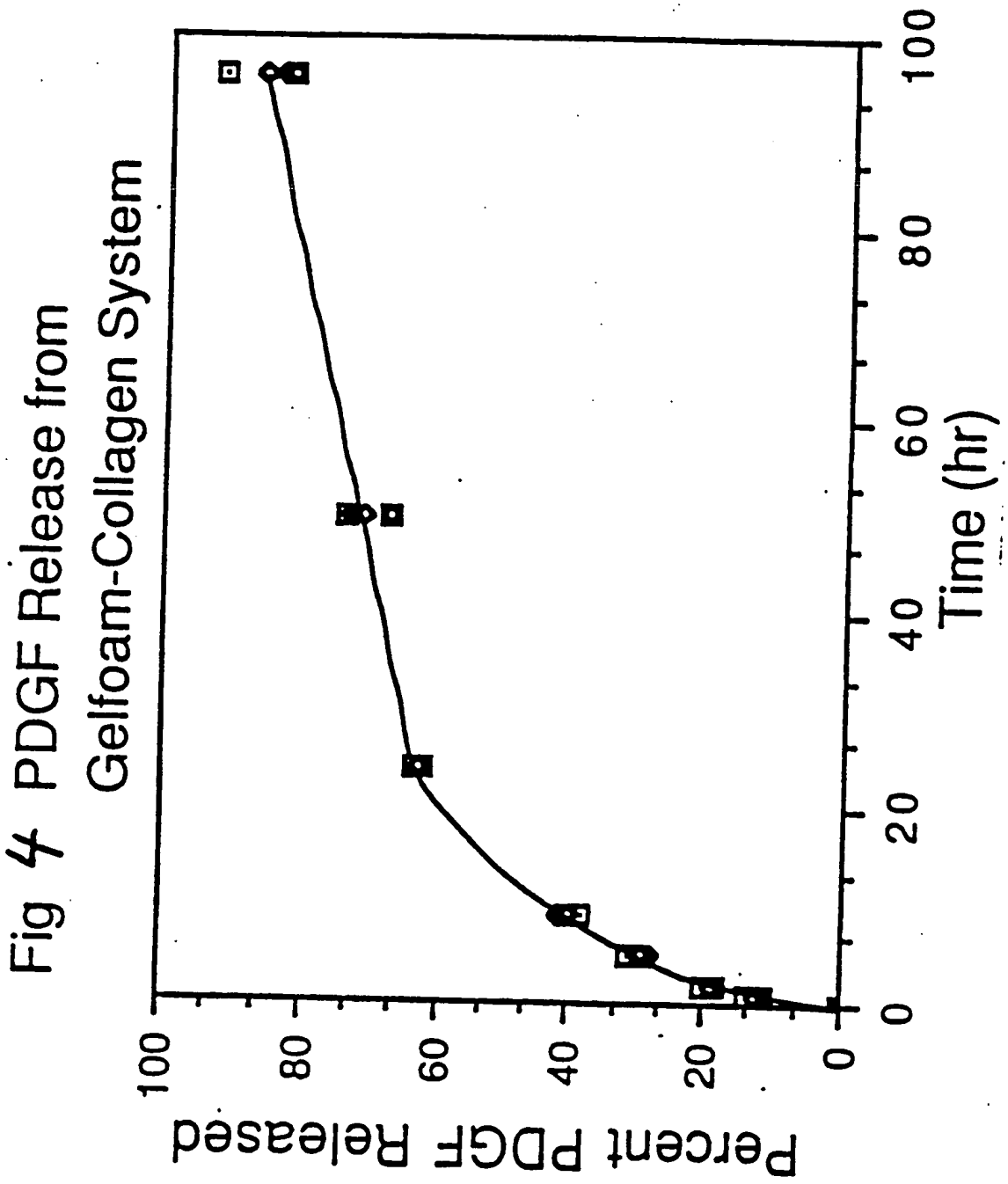


Fig 4 PDGF Release from Gelfoam-Collagen System

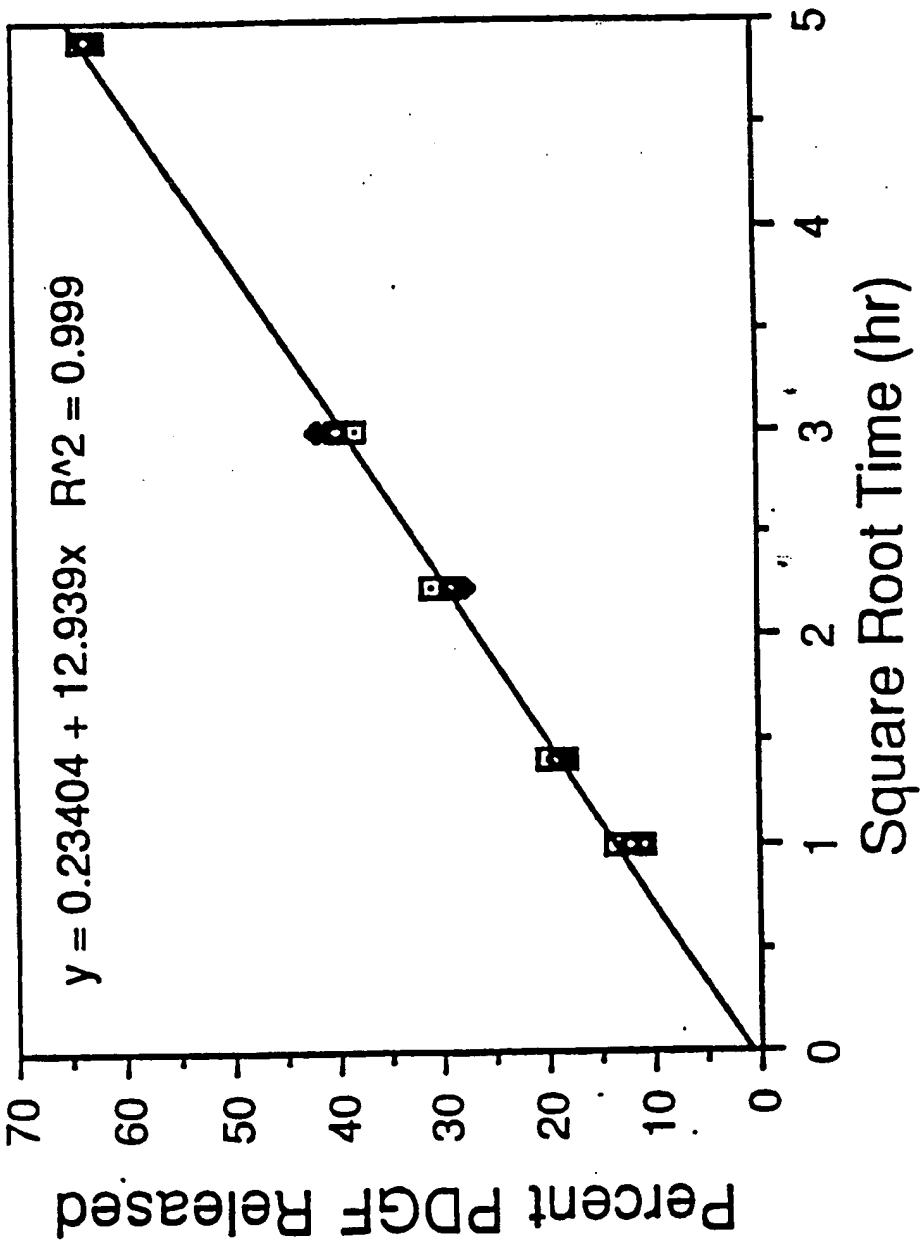


Fig 5 PDGF Release from Gelfoam-Collagen System

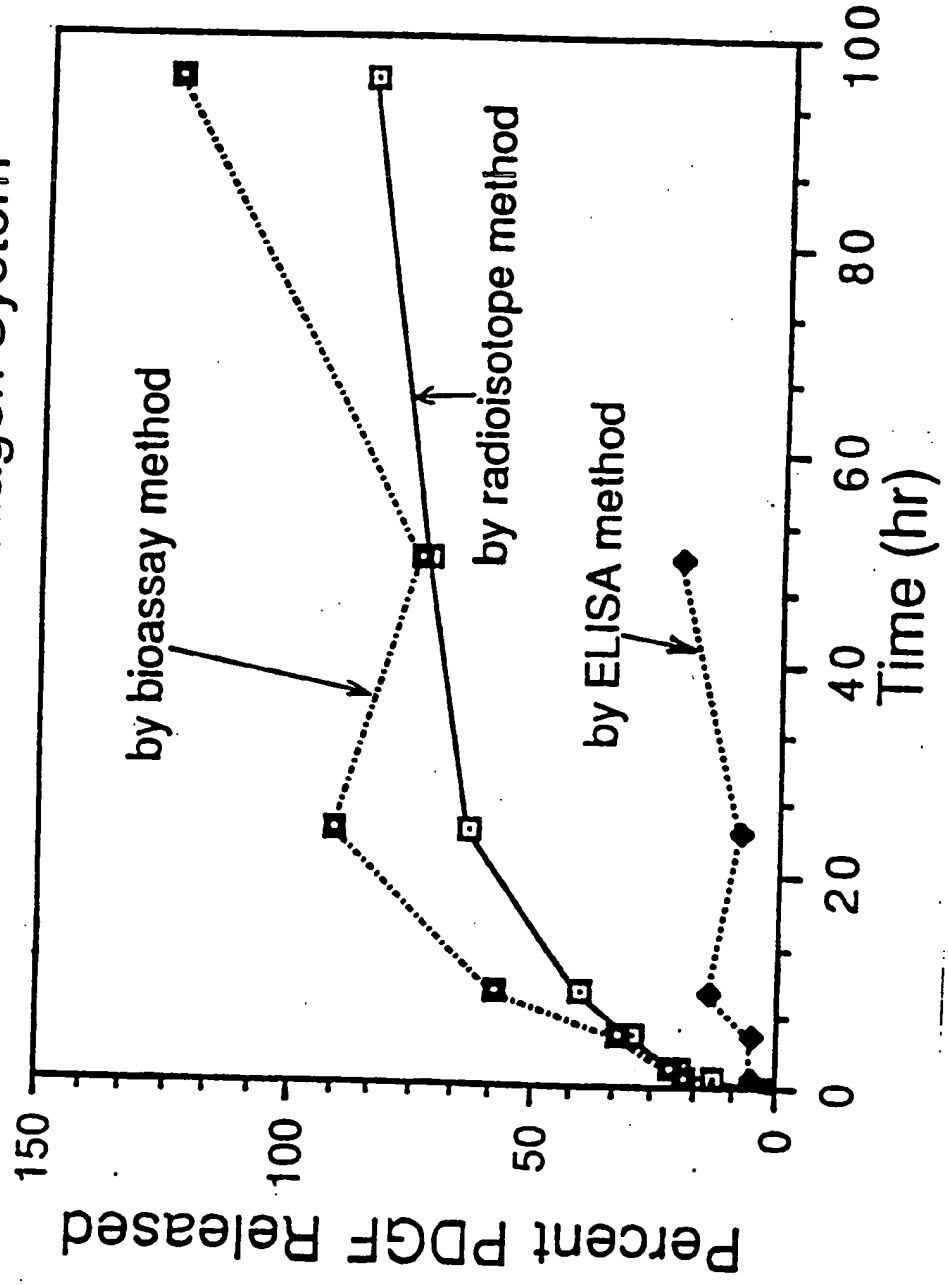


Fig 6 PDGF Release from Gelfoam-Collagen System

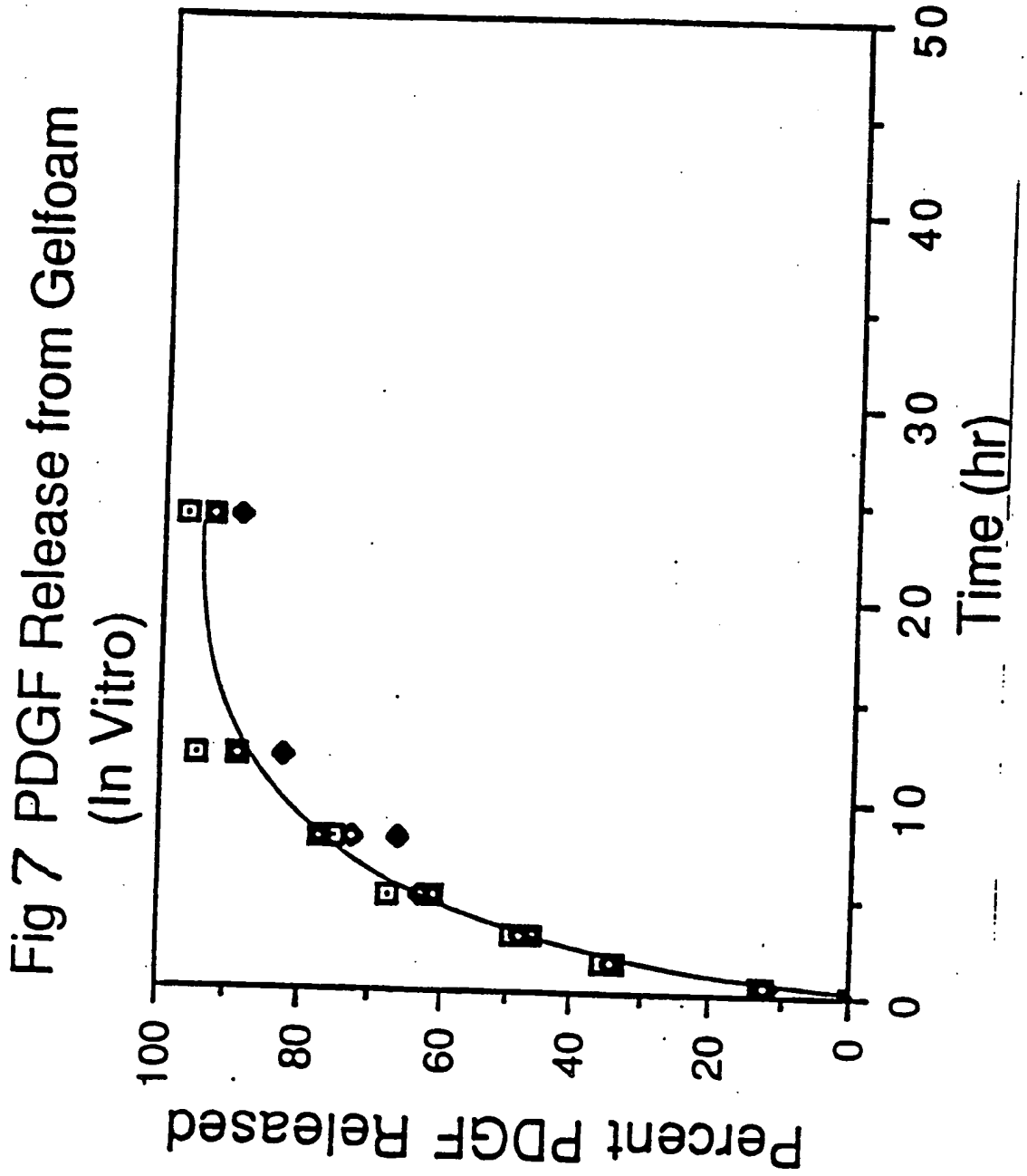


Fig 7 PDGF Release from Gelfoam (In Vitro)

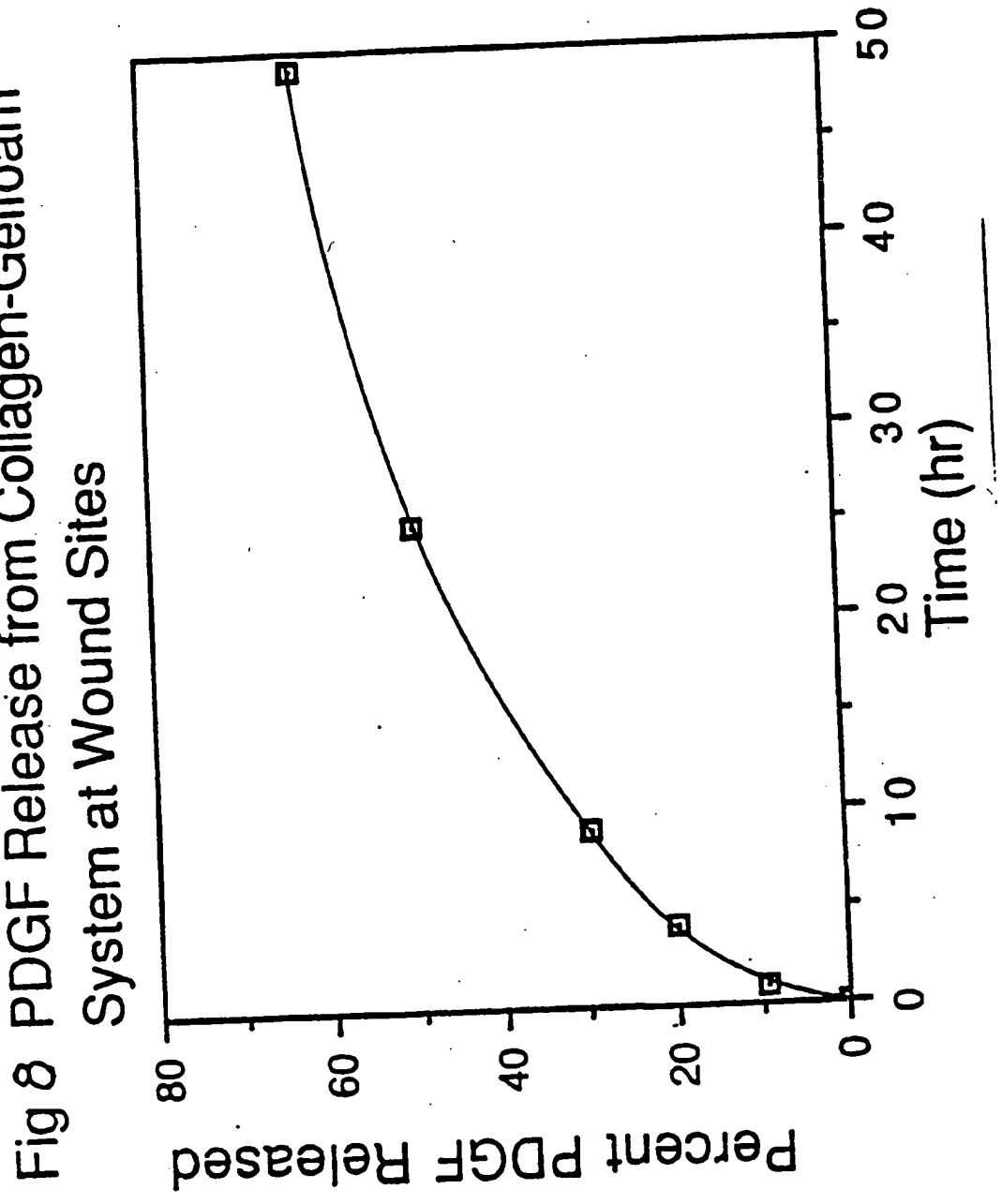


Fig 8 PDGF Release from Collagen-Gelfoam System at Wound Sites

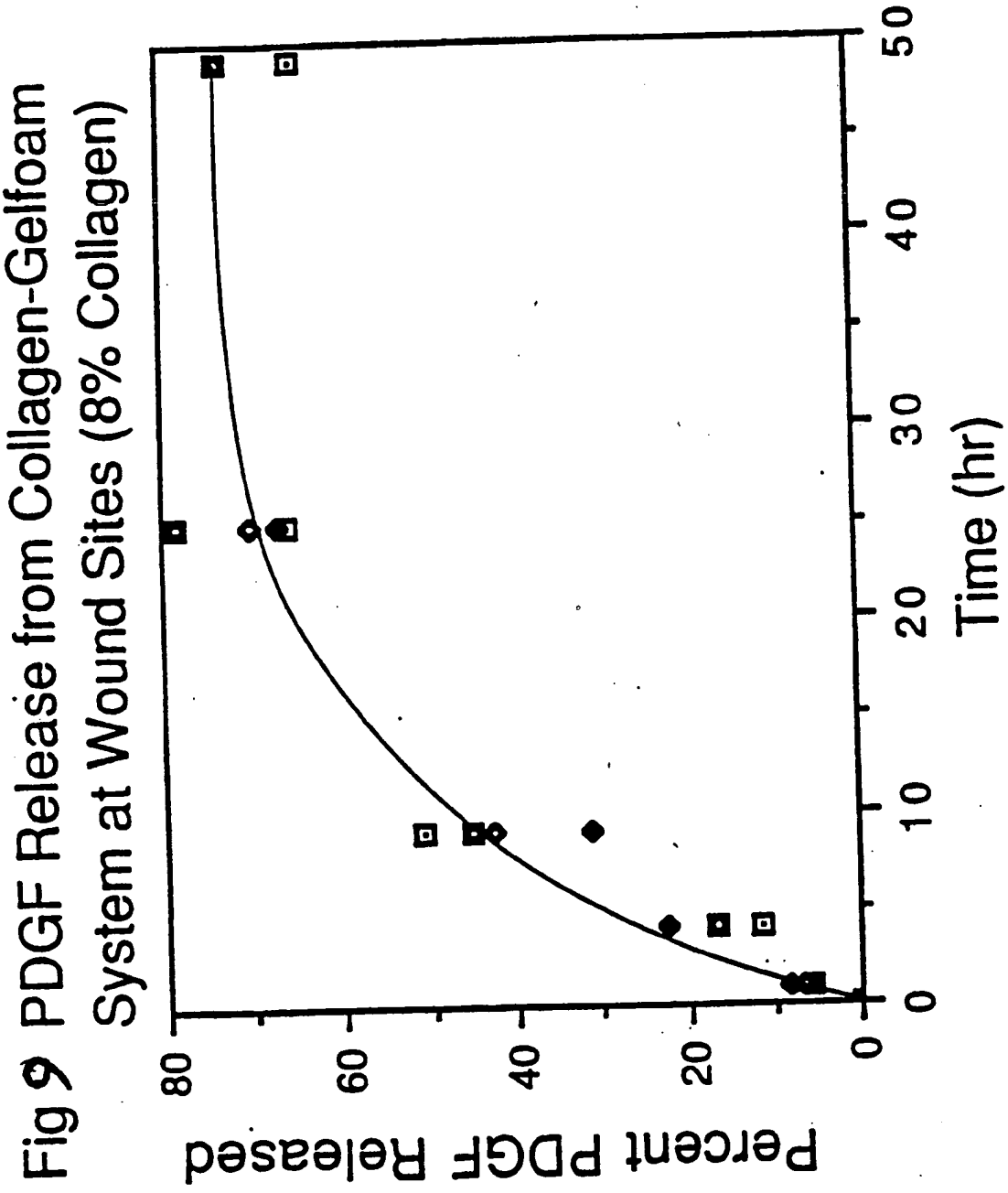
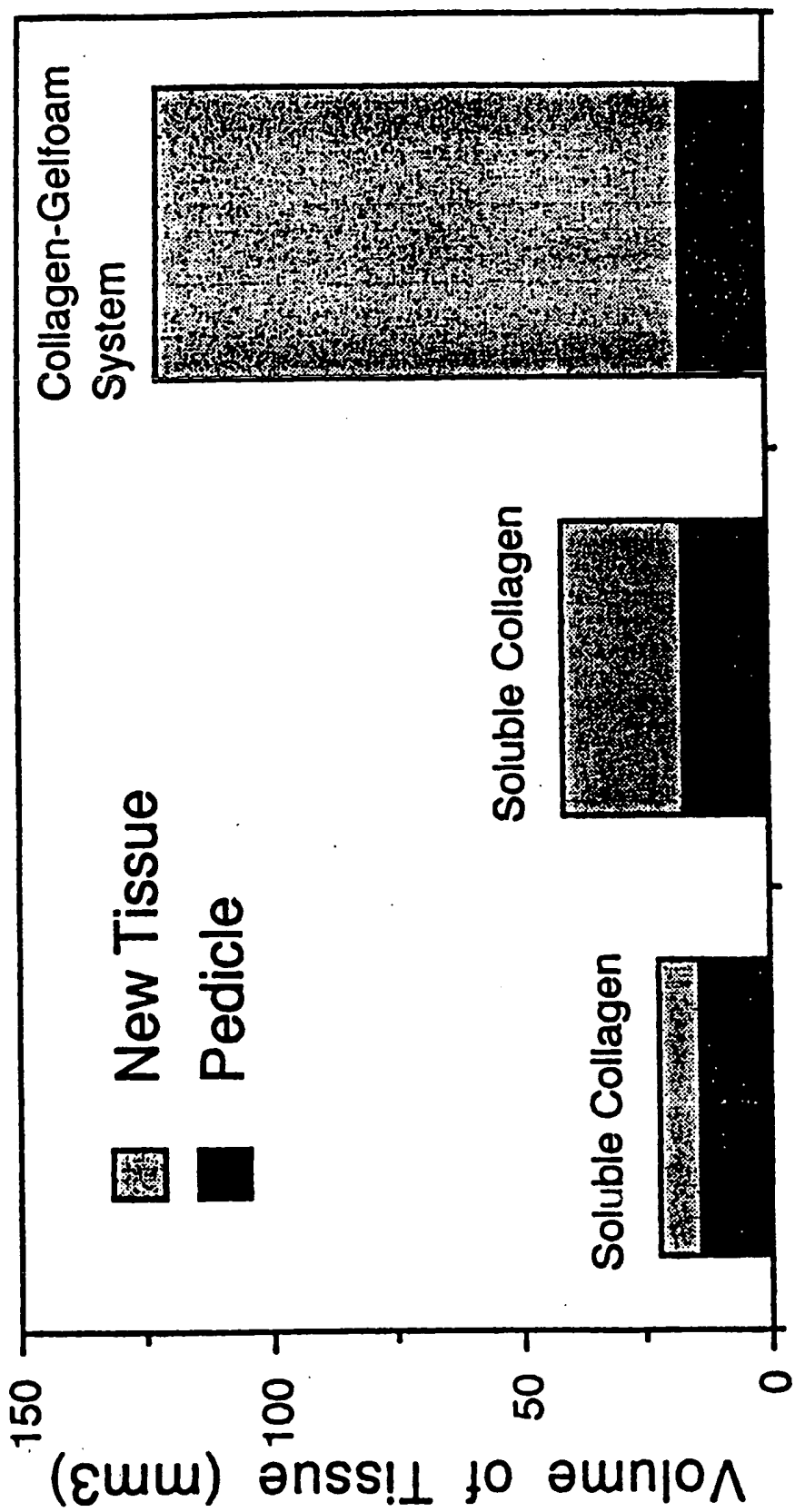


Fig 9 PDGF Release from Collagen-Gelfoam System at Wound Sites (8% Collagen)

Figure 10 Tissue Generation with PDGF



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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 93 30 3307

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 5)
X Y	WO-A-9 013 320 (FERROSAN A/S) * page 12, line 26 - line 35 * * page 15, line 4 - line 30 * * page 11, line 6 - page 12, line 17 * * claims *	1-3,6-7, 10 4-5,8-9, 11-18	A61L15/64 A61L15/44 A61K9/20 A61L15/32
Y,D	WO-A-9 000 060 (COLLAGEN CORPORATION) * page 3, line 5 - line 20 * * page 11, line 4 * * claims *	11-18	
Y	US-A-4 292 972 (J. PAWELCHAK) * column 5, line 38 - line 39 *	4-5	
Y,P	EP-A-0 518 697 (AMGEN INC) * page 4, line 26 - line 30 *	8-9	
A D	WO-A-8 504 413 (UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY) & US-A-4 841 962		

TECHNICAL FIELDS SEARCHED (Int. Cl. 5)

A61L
A61K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	20 JULY 1993	G.COUSINS-VAN STEEN

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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